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IMPACT OF REPLACING GRAPE POLYPHENOL WITH VITAMIN E ON GROWTH PERFORMANCE, RELATIVE ORGANS WEIGHT AND ANTIOXIDANT STATUS OF BROILERS

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ABSTRACT

Impact of replacing vitamin E with Grape Polyphenol (GPP) on growth performance, relative organs weight and antioxidant status of broilers was examined in the present study. A total of 280 Hubbard broiler chicks were randomly divided into 28 experimental units of 10 chicks each. A basal starter diet (control) was formulated having 22.5% CP, 3200 kcal/kg ME and 100-ppm vitamin E. The other dietary treatments were obtained by fortifying the basal diet with 25 (low GPP; LGPP), 50 (medium GPP; MGPP) and 75 ppm (high GPP; HGPP) GPP in place of vitamin E. Likewise, a finisher basal diet (20% CP and 3200 kcal/kg ME) and other dietary treatments were formulated having same proportions of GPP and vitamin E as in starter diets. Each dietary treatment was randomly assigned to seven replicate pens. Feed intake, weight gain and feed conversion ratio remained unaltered among different treatment groups during all growth periods. However, free radical scavenging activity in breast and leg muscles increased quadratically ($P<0.05$) with MGPP showing the highest antioxidant status ($P<0.05$). The organs like kidney and heart showed linear depression in weight by increasing GPP concentration while relatively lower weight of liver was observed in MGPP diet. In conclusion, replacing vitamin E with GPP in broiler diets did not exhibit difference in performance however, better antioxidant status was observed by addition of 50 mg/kg of GPP in the diet.

Key words: grape polyphenol, antioxidant status, immune response, organs histopathology, broiler.

INTRODUCTION

Broilers are being raised now days in high-density flocks to reduce housing, management and labor costs on one hand and to maintain optimal environment conditions on the other. However, higher density rearing of broiler flocks may result in severe effects on health and performance (Shanawany, 1988; Webster, 1990) and thus, requires very sound management and nutritional manipulations. One nutritional tactic is to fortify the broiler diets with micronutrients (Chew, 1996). However, this is the most controversial and debating issue as it might lead to over nutrition, toxicities and increased costs. To minimize such risks, it is required to explore new nutrients which when fed in excessive amounts, do not have health hazards.

Several researchers have successfully used various extracts from plants against many pathogens (Visintini *et al*., 2013). Phenolic compounds extracted from plants are widely used successfully due to their antioxidant functions and positive effects on performance (Selma *et al*., 2009; Dorri *et al*., 2012a, b). It has also been reported that the grape polyphenols (GPP) exhibit more antioxidant and thermo stability properties. Thus, they have not only shown synergistic effects with vitamin E (Gladine *et al*., 2007; Yildirim *et al*., 2011) but also have the potential to replace vitamin E as an antioxidant. However, higher levels of these polyphenols have been reported to depress growth rate in broilers (Lau and king, 2003; Hughes *et al*., 2005). There is some published data available regarding use of grape by-products such as grape seed extracts or grape pomace in poultry (Brenes *et al*., 2010; Viveros *et al*., 2011; Dorri *et al*, 2012 a, b) but very little data is available on the effect of replacing vitamin E with GPP. Therefore, the present study was conducted to examine the effects of replacing vitamin E with GPP on growth performance, relative organs weight and antioxidant status of broilers.

MATERIALS AND METHODS

Birds, Housing and Feeding Management: Two hundred and eighty day-old Hubbard broiler chicks were randomly divided into twenty-eight experimental units of 10 chicks each. Birds in each replicate were placed in separate pen measuring 5 x 2.5 feet. The birds were vaccinated against ND virus on day 7 and 28 and against IBD virus on day 9 and 21. The birds were kept under standard management conditions of temperature, light and ventilation. Fresh, clean drinking water was provided at ad-libitum to the birds round the clock.

Experimental Diets: A basal starter diet (control) was formulated having 22.5% CP, 3200 kcal/kg ME and 100 ppm vitamin E (DL-α-tocopheryl acetate). The other dietary treatments were obtained by fortifying the basal diet with 25 (low GPP; **LGPP**), 50 (medium GPP; **MGPP**) and 75 ppm (high GPP; **HGPP**) GPP (Grape PP concentrate, Provimi BV, AN Rotterdam, Netharlands) in place of vitamin E. Likewise, a finisher basal diet (20% CP and 3200 kcal/kg ME) and other dietary treatments were formulated having same proportions of GPP and vitamin E as in starter diets (Table 1). Each dietary treatment was randomly assigned to seven replicate pens. Starter and finisher diets were fed at ad-libitum from day 1 to 21 and day 22 to 35, respectively.

Data Collection and Analysis: Feed intake and weight gain were recorded at the end of each phase and this data was used to calculate feed conversion ratio (FCR). On 35th day, two birds form each replicate were randomly selected and slaughtered to collect breast and thigh tissue samples. These samples were stored in polyethylene zipper bags at -20^{o}C until analyzed for free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) test (Brand-Williams, 1995). A Sample (500 µL) having 100µL protein/mL was taken in a test tube. 2 mL freshly prepared 0.0012M DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol solution was added in tubes containing 100 µL volume of sample. Afterwards it was incubated (30 minutes) at room temperature in dark place. Ultraviolet/Violet rays' spectrophotometer (Uv-Vis Spectrophotometer Model# FEN Spec T70 made in United Kingdom) was used to check the absorbance of resultant mixture at 517 nm against blank and control. Heart, Kidney and liver samples were taken as a whole and their weight was recorded and grossly examined for any histo-pathological lesions, and normal color and consistency.

Statistical Analysis: Data collected were analyzed using the Analysis of Variance (ANOVA) technique in a Completely Randomized Design (Steel *et al*., 1998). Duncan's Multiple Range test was used to compare the means ($P<0.05$). Linear and quadratic regression analyses were also done to estimate the birds' response to various dietary treatments.

RESULTS

The replacement of vitamin E with GPP did not significantly affect the feed intake, weight gain and FCR of birds during any growth period (Table 2). However, free radical scavenging activity in breast and leg muscles increased quadratically ($P<0.05$), with MGPP showing the highest antioxidant status ($P<0.05$). The relative organs weight showed significant ($P<0.05$) results with kidney and heart showing linear depression by increasing GPP concentration while relatively lower weight of liver was observed in MGPP diet (Table 4). All these organs showed normal color with no lesions when grossly examined.

Table 1. Ingredient and nutrient composition of starter and finisher basal diets

Ingredients (%)	Starter diet	Finisher diet
Maize	42	44
Rice Tips	20	21
Corn Gluten 60%	3.16	3
Soybean Meal	23	20
Fish Meal	5	5
Limestone	1.17	1
Dicalcium Phosphate	0.38	0.37
Lysine Sulphate	0.29	0.20
DL-Methionine	0.90	0.80
Common Salt	1.6	1.13
Sunflower Oil	2.0	3.0
[1]Vitamin and mineral Premix	0.5	0.5
Total	100	100
Chemical Composition%		
Metabolizable Energy (MJ/kg)	13.40	13.40
Crude Protein	22.5	20.0
Crude Fiber	2.73	2.73
Calcium	1.0	0.90
Av.Phosphorus	0.65	0.44
Lysine	1.11	0.90
Methionine	0.55	0.40

[1]Supplied per kg of diet: Vitamin A (as retinyl acetate), 4360 IU; vitamin D3 (as cholecalciferol), 1000 IU; vitamin K (menadione sodium bisulfite), 2.8 mg; vitamin E (DL-α-tocopheryl acetate) 100 IU: Thiamine, 1.5 mg; Riboflavin, 6 mg; Niacin, 30 mg; Pantothenic acid, 14 mg; Pyridoxine, 1.5 mg; Cyanocobalamin, 0.016 mg; Folic acid, 1 mg; Biotin, 0.1 mg; Manganese, 0.6 gm; Zinc, 0.2 gm; Ferrous, 0.15 gm; Copper, 0.03 gm; Sodium chloride, 1.5 gm

Table 2. Growth performance of broilers fed diets containing different levels of vitamin E and GPP1

Parameters	Experimental diets[2]				SEM	Probabilities[3]	
	C	LGPP	MGPP	HGPP		Q	L
Starter phase (1-21 days)							
Feed Intake (g)	1002	983	965	992	16.6	NS	NS
Weight gain(g)	761	774	728	712	12.4	NS	*
Feed conversion ratio	1.31	1.27	1.33	1.39	0.05	NS	NS
Finisher phase (22-35 days)							
Feed Intake (g)	2034	2048	2045	2065	25.9	NS	NS
Weight gain(g)	1086	1077	1090	1133	29.8	NS	NS
Feed conversion ratio	1.87	1.90	1.87	1.82	0.04	NS	NS
Starter cum finisher phase (1-35 days)							
Feed Intake (g)	3036	3031	3010	3057	22.5	NS	NS
Weight gain(g)	1847	1851	1818	1845	26.3	NS	NS
Feed conversion ratio	1.64	1.64	1.63	1.67	0.02	NS	NS

a- d Means within a row with different superscripts differ significantly (P < 0.05)

[1]Mean of seven replicates with two birds from each replicate

[2]C: Control (100 ppm vitamin E + 0 ppm GPP), LGPP: Low grape polyphenols (75 ppm vitamin E + 25 ppm GPP), MGPP: Medium grape polyphenols (50 ppm vitamin E + 50 ppm GPP), HGPP: High grape polyphenols (25 ppm vitamin E + 75 ppm GPP)

[3]L and Q stand for linear and quadratic response to increasing GPP level, respectively

NS: Non-significant and *: Significant (P<0.05)

Table 3. Antioxidant activity in breast and leg muscles of broilers fed diets containing different levels of vitamin E and GPP[1.]

Parameter	Experimental diets[2]				SEM	Probabilities[3]	
	C	LGPP	MGPP	HGPP		L	Q
Free Radical Scavenging Activity (DPPH)							
Breast	72.22[a]	68.78[b]	62.10[d]	66.18[c]	0.337	*	*
Leg	71.80[a]	67.32[b]	61.93[d]	65.68[c]	0.263	*	*

a- d Means within a row with different superscripts differ significantly (P < 0.05)

[1]Mean of seven replicates with two birds from each replicate

[2]C: Control (100 ppm vitamin E + 0 ppm GPP), LGPP: Low grape polyphenols (75 ppm vitamin E + 25 ppm GPP), MGPP: Medium grape polyphenols (50 ppm vitamin E + 50 ppm GPP), HGPP: High grape polyphenols (25 ppm vitamin E + 75 ppm GPP)

[3]L and Q stand for linear and quadratic response to increasing GPP level, respectively

NS: Non-significant and *: Significant (P<0.05)

Table 4. Relative organs weights of broilers fed diets containing different levels of vitamin E and GPP[1]

Items	Experimental Diets[2]				SEM	Probabilities[3]	
(mg/Kg)	C	LGPP	MGPP	HGPP		L	Q
Heart	17.1429[a]	12.1429[b]	12.1429[b]	11.4286[c]	2.08248	*	NS
Kidney	23.5714[a]	17.1429[ab]	15.7143[ad]	15.00[ac]	1.72516	*	NS
Liver	61.42[a]	59.28[b]	51.42[ab]	52.14[ac]	4.41280	*	*

a- d Means within a row with different superscripts differ significantly (P < 0.05)

[1]Mean of seven replicates with two birds from each replicate

[2]C: Control (100 ppm vitamin E + 0 ppm GPP), LGPP: Low grape polyphenols (75 ppm vitamin E + 25 ppm GPP), MGPP: Medium grape polyphenols (50 ppm vitamin E + 50 ppm GPP), HGPP: High grape polyphenols (25 ppm vitamin E + 75 ppm GPP)

[3]L and Q stand for linear and quadratic response to increasing GPP level, respectively

NS: Non-significant and *: Significant (P<0.05)

DISCUSSION

The performance results indicated that GPP did not depress the growth performance of broilers when incorporated in diet up to 75mg/ Kg in place of vitamin E. This might be due to low concentration of GPP in the experimental diets. In present study, 3.8% total extractable polyphenols were added @ 75 mg/Kg of diet at maximum, which is too low to depress performance of birds. Previously, growth depression results have been reported due to addition of polyphenols in poultry diets (Lau and king, 2003; Hughes *et al*., 2005). In those studies, 90.2% total extractable polyphenols were added to diet @ 30g/Kg, and this concentration was high enough to depress the growth of birds. Results of present study are consistent with the findings of Goni *et al*. (2007) who did not report growth depression in birds fed diet supplemented with grape pomace concentrate up to 30 g/Kg. Similarly, Brenes *et al*. (2008, 2010) and Vivors *et al*. (2011) did not report growth depression in broilers when fed diet supplemented with grape pomace, grape seed extract and GPP, respectively.

The MGPP group showed better antioxidant activity as compared to all other groups. This higher antioxidant activity may be the result of higher proanthocyanidins compounds in GPP. It has been reported that grape seed proanthocyanidins show 50 times more antioxidant property than vitamin E (Konowalchuk and Spiers, 2009). These results are in agreement with the reports of Brenes *et al.* (2010) who reported a linear increase in antioxidant activity in grower and finisher diets as well as in excreta of birds fed on grape seed extracts. Goni *et al*. (2007) and Brenes *et al*. (2008) also reported higher antioxidant activity in chicken fed diets supplemented with grape pomace and grape seed extract, respectively.

Regarding the relative organs weight, there is no reference in the literature in relation to GPP effects on heart, liver and kidney weights. However, Brenes *et al*. (2010) reported a depression in intestinal length in birds fed on diet containing grape seed extracts. They also reported increased spleen weight along with immune boosting action of these extracts as main cause of increased spleen weight. At this time, we do not have any explanation regarding depression in relative organs weight.

In conclusion, replacement of vitamin E with GPP showed better antioxidant status of birds without any negative effects on growth performance. In general, optimum results were obtained in broilers fed diet containing 50 mg/Kg GPP in the present study. Further research is needed to determine the effect of GPP on broiler performance.

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